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ORIGINAL ARTICLE

Validated stability-indicating high performance thin layer chromatographic method for determination of Ivabradine hydrochloride in bulk and marketed formulation: An application to kinetic study

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Abstract A sensitive, selective, precise and accurate stability-indicating high-performance thin layer chromatographic method for analysis of Ivabradine hydrochloride (IH) an anti anginal agent, both as a bulk drug and in formulations was developed and validated according to ICH guideline. Densitometric analysis of IH was carried out in the absorbance mode at 287 nm using ethyl acetate: 0.389 M ammonium acetate in methanol (1:5, v/v) as solvent system. This system was found to give compact spots for IH at an R_f value of 0.36 ± 0.01. Moreover, IH was subjected to acid and alkali hydrolysis, oxidation, accelerated humidity/temperature, wet heat treatment, and photo degradation. The drug undergoes degradation under mainly acidic and basic conditions. Also the degraded products were well resolved from the pure drug with significantly different R_f values. Linearity was found to be in the range of 1200–2800 ng/band. The LOD and LOQ for IH were 255.86 ng/band and 775.33 ng/band, respectively. "Bartlett's test" and "Lack of fit" applied on peak area for linearity, additionally proved validity of the developed method. Good accuracy and precision were obtained as revealed from %RSD value less than 2. Similarly, no interference was observed from common excipients in tablet formulation as well as degradation product, indicating specificity of the method. As the method could effectively separate the drug from its degradation product, it

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can be employed as a stability-indicating one. Moreover, proposed method was also utilized to investigate the kinetics of acidic degradation process at different temperatures and first order rate constant, half-life, shelf-life and activation energy were calculated.

1. Introduction

Ivabradine hydrochloride (IH), chemically 3-[3-({[(7S)-3, 4dimethoxybicyclo[4.2.0]octa 1,3,5-trien-7yl]methyl}(methyl)amino) propyl]-7,8dimethoxy-2,3,4,5-tetrahydro-1*H*-3 benzazepin-2-one (Fig. 1), is an anti-ischaemic agent with specific negative chronotropic action.[1] IH is a specific heart rate lowering agent, acting by reducing the rate of pacemaker activity in the sinoatrial node. Within the sinoatrial node, IH is a selective inhibitor of I_f, an important current involved in generating the early phase of spontaneous diastolic depolarization in pacemaker cells, thereby reducing the frequency of action potential initiation and lowering the heart rate.[2] IH thereby prolongs the duration of diastole, so as to improve the balance between myocardial oxygen supply and demand as well as coronary perfusion.[3] In context to this, IH decreases oxygen consumption thereby preventing symptoms and reducing morbidity and mortality in patients with coronary artery disease and angina.[4]

Literature reports, analysis of IH in human plasma and urine, rat and dog plasma by high performance liquid chromatography with fluorescence detection and liquid chromatography–mass spectrometry method.[5,6] Moreover, determination of IH in marketed formulation by simple HPLC and UV spectrophotometric methods, dissolution profile study of immediate release tablet formulation of IH by HPLC are also available in literature.[7–10] Also, stability indicating HPLC method has been reported for the determination of IH in bulk and dosage form.[10] Literature reviewed reveals no information related to the stability-indicating methodology by high performance thin layer chromatography (HPTLC) for the determination of IH in pharmaceutical dosage forms. Accordingly, the purpose of the present study is to put ICH recommendations into practice by subjecting IH to a variety of suggested stress test conditions along with kinetic study to establish inherent stability of the drug and to develop the validated stability indicating HPTLC assay.

According to the International Conference on Harmonization (ICH) guidelines, requirements for establishing SIMs have become more clearly mandatory.[11] Moreover, kinetic studies and accelerated stability experiments play a significant role for solving problems encountered in quality control and to predict the expiry dates of pharmaceutical products. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as temperature, light, oxygen, pH and moisture. Stress testing can help in identifying degradation products and provide important information about the intrinsic stability of drug substances.[12]

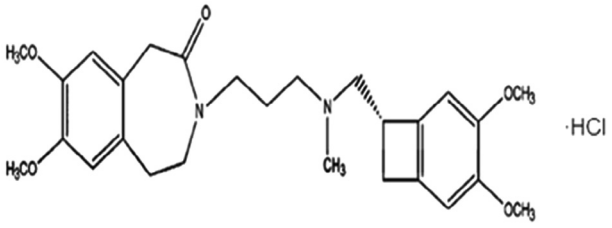

Figure 1 Chemical structure of Ivabradine hydrochloride.

Although LC procedures are accurate and effective means for analysis, they are time consuming; in addition, one of the major drawbacks is the use of large amounts of solvents and expensive instrumentation. As a consequence, rapid, efficient, and inexpensive analytical procedures that meet the continuous need for high-throughput assays of the drugs in quality control laboratories are highly demandable. Introducing TLC into pharmaceutical analysis represents a major step in terms of quality assurance. Today, TLC and HPTLC are rapidly becoming routine analytical techniques due to their several advantages over other methods.[13] The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering the analysis time, sample clean up and cost per analysis.[14] Also, HPTLC plates are pre-coated with smaller size particles, have narrow particle size distribution, and possess smooth surfaces and thinner layers. Other characteristics of HPTLC compared with TLC include smaller sample volume, economy, faster separation, more reproducible results and lower detection limits. Furthermore, it provides processing of samples and standards at the same time and so could be used for the quantitative determination of such chemical compounds with high degree of accuracy and precision. Thus, separation and quantification can provide results that are either superior or comparable with other analytical methods such as HPLC.[15]

An ideal stability indicating method hence was developed by HPTLC that is capable of quantifying IH and can also resolve IH from its degradation products. Furthermore, the proposed method was used to study the degradation kinetics profile of IH under acidic condition at different temperature and degradation kinetic parameters like activation energy, degradation rate constant, t_{90} (where 90% of original concentration of drug is left) and t_{50} (half-life) were computed from Arrhenius plot. The proposed stability indicating method is simple and allows rapid analysis for stability studies and quality control analysis of drug in bulk and dosage form.

2. Experimental

2.1. Materials

Pharmaceutical grade of Ivabradine hydrochloride (IH) was kindly supplied as a gift sample by Biocon Ltd., Andhra Pradesh, India, used without further purification. All solvents and chemicals used were of analytical grade, purchased from Merck Specialities Pvt. Ltd., India. Marketed tablet dosage forms used in this study were IVABRAD5

and 7.5 (Ind-Swift Ltd.) and IVABID5 and 7.5 (Abbott Healthcare Pvt. Ltd.) procured from local market.

2.2. Instrumentation

Microsyringe (Linomat syringe 659.0014, Hamilton-Bonaduz Schweiz, Camag, Switzerland), pre-coated silica gel $60F_{254}$ aluminium plates (10×10 cm, 100 μm thickness; Merck, Darmstadt, Germany), Linomat 5 applicator (Camag, Switzerland), twin trough chamber (10×20 cm; Camag, Switzerland), UV chamber (Camag, Switzerland), TLC scanner IV (Camag, Switzerland), winCATS version 1.4.6 software (Camag, Switzerland) were used in the study.

2.3. Preparation of standard stock solution

A stock solution of IH was prepared by weighing accurately 10 mg of drug followed by dissolution and dilution in distilled water to obtain a concentration of 100 μg/ml. This stock solution was appropriately diluted to make working standard solution as and when necessary.

2.4. Chromatographic procedure

The standard solutions of different concentrations were spotted in the form of bands having a band width of 6 mm with a microsyringe on pre-coated silica gel aluminium Plate $60F_{254}$, using a Camag Linomat 5sample applicator. Linear ascending development was carried out in a twin trough glass chamber. The mobile phase consisted of ethyl acetate: 0.389 M ammonium acetate in methanol (1:5, v/v). The optimized chamber saturation time before chromatographic development was 20 min at room temperature (25 °C ± 2). The length of chromatographic run was 8 cm which took an average of 12 min to develop. Subsequent to the development; HPTLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed using a Camag TLC scanner IV with winCATS software. All measurements were made in the reflectance–absorbance mode at 287 nm, slit dimension (6.00×0.30 mm, micro), scanning speed 20 mm/s, data resolution 100 μm/step. The source of radiation was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentration of the drug was determined from the intensities of diffusely reflected lights. Evaluation was via peak areas with linear regression analysis.

2.5. Calibration curve

Different volumes of the above standard solution were applied and linear relationship between peak area and concentration of the drugs was evaluated over the concentration range of 1200–2800 ng/band by making five replicate measurements.

2.6. Method validation

The method was validated in accordance with ICH guidelines Q2 (R1) for evaluation of linearity, precision, accuracy, LOD, LOQ, specificity and robustness.[16]

2.6.1. Precision

Precision of the developed method was evaluated by performing repeatability on same day and intermediate precision studies on different days and peak area measured was expressed in terms of percent relative standard deviation (%RSD). Repeatability of sample application and measurement of peak area were determined by six replicate applications of same spot (1600 ng/band). Repeatability and intermediate precision were carried out by performing three replicates of three different concentrations (1200, 2000 and 2800 ng/band).

2.6.2. Accuracy

Accuracy of the method was ascertained by performing recovery at three levels (80%, 100% and 120%), by spiking with IH standard (960, 1200 and 1440 ng/band) to the dosage form (1200 ng/band) by standard addition method. Recovery studies were performed in triplicate.

2.6.3. Limit of detection (LOD) and limit of quantitation (LOQ)

As per ICH guideline, limit of detection and quantification of the developed method were calculated from the standard deviation of the *y*-intercept and slope of the calibration curve of IH using the formula,

$$\text{Limit of detection} = 3.3 * \sigma / S \quad (1)$$

$$\text{Limit of quantitation} = 10 * \sigma / S \quad (2)$$

where, "σ" is standard deviation of response"S" is slope of calibration curve

2.6.4. Robustness

The effect of deliberate variations in method parameters like the composition of the mobile phase ethyl acetate: 0.389 M ammonium acetate in methanol (0.8:5.2, v/v) and (1.2:4.8, v/v), saturation time (15 and 25 min), development distance (7 and 9 cm), spot scanning time interval (developed plate was scanned after 1, 2, 3, 4, 5, and 6 h),wavelength scan (285 and 289 nm), time from spotting to chromatography (0, 20, 40 and 60 min), mobile phase volume (6, 12 and 18 ml) was evaluated. The effect of these changes on both the R_f values and peak areas was evaluated by calculating the (%RSD) for each parameter.

2.6.5. Specificity

The specificity of the method was ascertained by comparing the samples of tablet formulation with standard drug. The band for IH in sample was confirmed by comparing the R_f values and peak purity spectra with that of standard. The peak purity of IH was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) position of the band. The specificity of the method was also confirmed by performing forced degradation studies followed by comparison of R_f value and peak purity spectra with that of standard.

2.7. Analysis of tablet dosage form

To determine the concentration of IH in tablet dosage form (label claim: 5 and 7.5 mg per tablet), the contents of 20 tablet were accurately weighed, their mean weight was determined and finely powdered in a glass mortar. An accurately weighed powder, equivalent to 10 mg of IH was weighed and transferred into a 100 ml volumetric flask containing 50 ml

distilled water, followed by sonication for 30 min and further dilution up to mark with distilled water. The resulting solution was filtered through whatman filter paper No. 42. Twelve microlitres of the filtered solution (1200 ng/band) was applied on the TLC plate followed by development and scanning as described in Section 2.2. The analysis was repeated in triplicate.

2.8. Forced degradation studies

To evaluate the stability indicating property of the developed HPTLC method, IH was subjected to forced degradation conditions according to following procedures like acid/base hydrolysis, oxidation, wet heat, temperature/humidity, dry heat and photo-degradation followed by development and scanning under optimized chromatographic conditions. The forced degradation in acid, base and hydrogen peroxide was performed in the dark, in order to exclude the possible degradative effect of light on the drugs.[17–21]

(a) 1000 µg/ml solution of IH was refluxed at 70 °C for 45 min in different conditions: acid hydrolysis (0.1 and 1 M hydrochloric acid separately), base hydrolysis (0.1 and 1 M sodium hydroxide separately), oxidation (0.3% and 3% hydrogen peroxide separately) and wet heat degradation were done.
(b) 10 mg of IH powder was subjected to various conditions: dry heat degradation (in an oven at 70 °C for 24 h), photo-degradation (exposed to UV light in UV chamber and sunlight for 24 h) and effect of temperature-humidity (in stability chamber at 40 °C and 75% RH for 24 h).

2.9. Degradation kinetic investigation

To study the acid induced degradation kinetics of IH, final concentration of IH standard solution was 2 mg/ml in 1 M hydrochloric acid. This flask was refluxed at 70, 80, 90 and 100 °C temperatures. At specified time intervals, the content of the flask was quantitatively transferred to 25 ml volumetric flask and further neutralized to give a final concentration of 1 mg/ml. From this neutralized solution, 2 µl (2000 ng/band) was applied on HPTLC plate and development was carried out under optimized chromatographic conditions. Each experiment was repeated three times at each temperature and time interval and the concentration of the IH remaining after degradation was calculated. Further calculations were done to obtain degradation rate constant, half-life, shelf-life and activation energy from Arrhenius plot. Furthermore, in situ UV densitometric scanning of both IH and degradation product was performed on HPTLC plates. Moreover, DP was also separated by scrapping from the HPTLC plate and IR spectra and Mass spectra of both IH and DP were obtained.

2.10. Statistical analysis

Statistical parameters like SD, %RSD were computed by using MS Excel. Bartlett's test and test for Lack of fit were applied on the data of areas of linearity for evaluation of homoscedasticity of variance and deviation from linearity.[22]

3. Results and discussion

3.1. Development of optimum mobile phase

In order to develop stability indicating method, pure drug and its degraded product were applied on plates and different solvents, alone and its combinations were tried in different ratios. IH is a salt form, hence preliminary study was initiated with different polar solvents with differing ratios (methanol: water; 1:1, v/v) but retention factor was found to be less with asymmetric peak shape. Acetic acid was hence added to increase peak symmetry and R_f but, IH was unstable in acetic acid. Hence, various other solvents were tried like methanol: ethyl acetate: ammonia that gave high R_f value with very broad and diffused peak. Toluene was added to above mobile phase to decrease the R_f value but spot lacks compactness and was less persistent. Alternatively, ammonia when replaced with different concentrations of ammonium acetate salt was found to separate both IH and its degradation product with an optimum R_f value and very sharp narrow peak. The mobile phase finally optimized for study was 0.3896 M ammonium acetate in methanol:ethyl acetate in ratio of 5:1, v/v; resulting in R_f value of 0.36 and 0.18 for IH and its degradation product, respectively (as shown in Fig. 4). Sharp and well defined symmetrical peaks were obtained when the chamber was saturated with mobile phase for 20 min at room temperature and scanned at 287 nm on HPTLC plates.

3.2. Calibration curve

IH showed good correlation over a concentration range of 1200–2800 ng/spot with respect to peak area. The linearity of calibration curve and adherence of system to Beer's law were evaluated by high value of correlation coefficient. Further linearity was validated by applying "Bartlett's test" confirming homoscedasticity of variance that was exemplified by χ^2 value less than the tabulated value. Moreover, linearity was also evaluated by "Lack of fit" where deviation of peak area in terms of F ratio was less than the tabulated one (Table 1).

3.3. Validation of method

3.3.1. Precision

%Relative standard deviation for repeatability of sample application and measurement of peak area was found to be 0.88% and 0.96%, showing system precision. %RSD value reveals that the proposed method provides acceptable intra and interday variations as shown in (Table 1).

3.3.2. Accuracy

The proposed method when evaluated for accuracy in terms of percent recovery at three levels (80%, 100% and 120%), showed percentage recovery at all three levels in the range of 98–102%, suggesting suitability of method to perform routine drug analysis (Table 1).

3.3.3. Limit of detection (LOD) and limit of quantitation (LOQ)

Limit of detection and Limit of quantitation of developed method were found to be 255.86 and 775.33 ng/band respectively, indicating acceptable sensitivity of the method.

Table 1 Analytical parameters using HPTLC method.

Parameter	IH
Linearity range (ng/band)	1200–2800
Correlation coefficient (r^2)	0.9956 ± 0.0023
Slope ± SD[a](S_a)	2.2502 ± 0.0957
Confidence limit of slope	2.1315–2.3685
Intercept ± SD[a] (S_b)	2756 ± 174.4648
Confidence limit of intercept	2539.8756–2972.1244
Limit of detection (ng/band)	255.86
Limit of quantitation (ng/band)	775.33
Bartlett's test[b]	0.0365
Lack of fit[c]	1.518
Precision (%RSD)[d]	
Repeatability	0.67–1.14
Intermediate precision	1.02–1.44
Accuracy (% recovery)[e]	98.80–101.55

[a] Average of five determinations.
[b] Calculated value less than tabulated value, 9.488 at 95% confidence interval.
[c] Calculated F value less than tabulated value, 5.92 at 95% confidence interval.
[d] Average of three determinations for each concentration.
[e] Average of three determinations at each level.

3.3.4. Robustness

Deliberate change in different parameters like mobile phase composition, saturation time, development distance, spot scanning time interval, wavelength scan, time from spotting to chromatography and mobile phase volume showed %relative standard deviation of peak area less than 2%, indicating that none of these variables significantly affects the performance of proposed method, thereby confirming robustness of the method (Table 2).

3.3.5. Specificity

The marketed formulation and degradation samples using the developed method, showed peak at R_f value of 0.36 for IH that was found to be at the same R_f value for IH standard by comparison of chromatograms. The peak purity of IH in both marketed formulations and degradation samples when evaluated by comparing the spectra at peak start, peak apex and peak end positions of the spot, showed good correlation i.e., $r(S, M) = 0.9999$ and $r(M, E) = 0.9997$, indicating specificity in the presence of excipients (Fig. 2). Similarly peak purity of IH in degradation sample also showed good correlation i.e., $r(S, M) = 0.9985$ and $r(M, E) = 0.9810$, indicating specificity in the presence of degradation product (Fig. 3).

3.4. Analysis of marketed tablet formulation

The marketed formulation IVABRAD 5 and 7.5 and IVABID 5 and 7.5 when analysed in triplicate using the developed method, showed only one peak at R_f value of 0.36 for IH in the chromatogram of tablet sample hence indicating no interference of the excipients present in the tablet formulation. The content of IH found in IVABRAD 5 and 7.5 was in the range of 99–100% and for IVABID 5 and 7.5 in the range of 98–102%, which proves applicability of the developed method in routine analysis of pharmaceutical dosage form (Table 3).

3.5. Stock solution stability

The stability of IH in solution was evaluated by storing the stock solution at room temperature for 24 h. An appropriate volume of stock solution was applied at time point of 0, 1, 3, 6, 10, 24 h and development was done under optimized condition. After development, densitogram was evaluated for additional peaks (if any) but there was no indication of IH instability in solution.

3.6. Stability indicating property

The suitability of the proposed method for estimation of IH in the presence of its degradation product was confirmed by performing forced degradation study under various conditions. The content of IH remaining in terms of % recovery was calculated. IH recovery in 0.1 and 1 M hydrochloric acid was ranging from 98% to 99.5% and 94% to 96% respectively. Similarly drug recovery in 0.1 and 1 M sodium hydroxide was ranging from 98% to 99% and 96% to 98% respectively.

Table 2 Robustness study of the HPTLC method for determination of IH.

Parameters	SD of area	RSD(%)	R_f value
1. Mobile phase composition[a]	1.53	1.51	
(EA:AA: 1.2:4.8)			0.36 ± 0.01
(EA: AA: 0.8:5.2)			0.35 ± 0.01
2. Saturation time[a] (20 ± 5 min)	0.21	0.21	0.35 ± 0.01
			0.36 ± 0.01
			0.36 ± 0.01
3.Development distance[a] (8 ± 1 cm)	1.41	1.43	0.36 ± 0.01
			0.36 ± 0.01
			0.36 ± 0.01
4. Spot scanning time interval[b] (1, 2, 3, 4, 5, and 6 h)	1.13	1.09	0.37
5. Wavelength scan[b] (287 ± 2 nm)	1.83	1.84	0.37
6. Time from spotting to chromatography[a] (0, 20, 40 and 60 min)	1.49	1.53	0.36 ± 0.01
7. Mobile phase volume[a] (12 ± 6 ml)	1.71	1.75	0.36 ± 0.01

[a] Average of three determinations for concentration of 2500 ng/band.
[b] Average of three determinations for five different concentrations 1200, 1600, 2000, 2400 and 2800 ng.

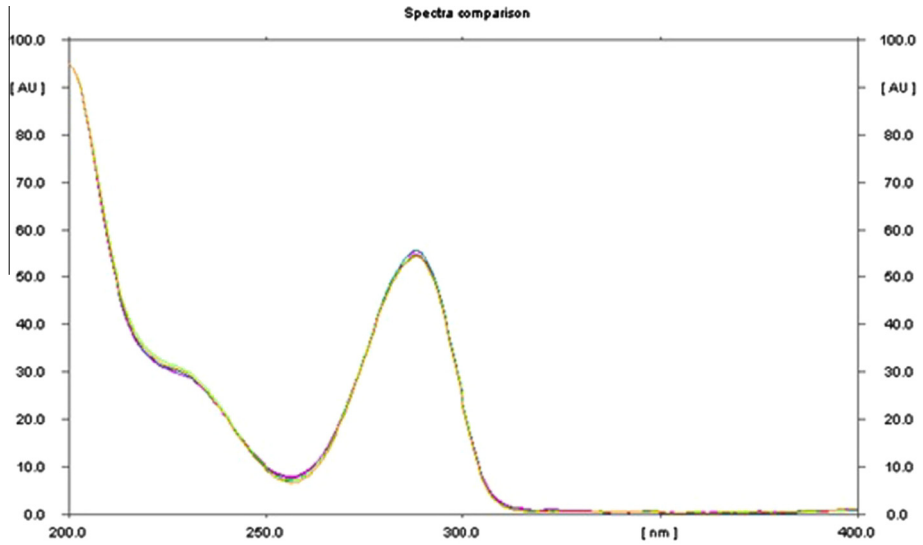

Figure 2 In situ UV overlain spectra of IH standard and tablet dosage form, (——) = Abbott 7.5 mg, (——) = Abbott 5 mg, (——) = IH standard, (——) = Ind swift 5 mg, (——) = Ind swift 7.5 mg.

This difference in drug recovery indicates that acidic degradation is slightly more pronounced than alkaline degradation. Chromatograms of both acidic and alkaline conditions showed additional peak of degradation product at an R_f value of 0.18 suggesting that degradant is common to both acid and base hydrolysis (Fig. 4).

Chromatogram of IH in all other conditions like: oxidation by hydrogen peroxide, dry heat and wet heat degradation, accelerated temperature and humidity condition showed no additional peak except IH at 0.36 ± 0.01, suggesting stability of IH in different conditions for specified period of time. In addition to this, colour of IH standard changed from white to light brown when exposed to UV light and sunlight for 24 h but degradation was not observed indicating no chemical changes in IH.

3.7. Degradation kinetic study

IH under stress conditions resulted in a gradual decomposition at all the selected temperatures (70, 80, 90 and 100 °C). Since the degradation was performed with a large excess of solvent, the degradation of IH followed pseudo-first-order kinetics with a linear relationship between log percentage of IH remaining with respect to time showing good correlation co-efficient (Fig. 5). Pseudo first-order is the term used when two reactants are involved in the reaction but one of them is in such a large excess that any change in its concentration is negligible compared with the change in concentration of the other reactant (drug). From slope of straight line obtained, first order rate constant (K), half-life ($t_{1/2}$) and shelf life (t_{90}) were calculated at each temperature for acidic degradation processes.

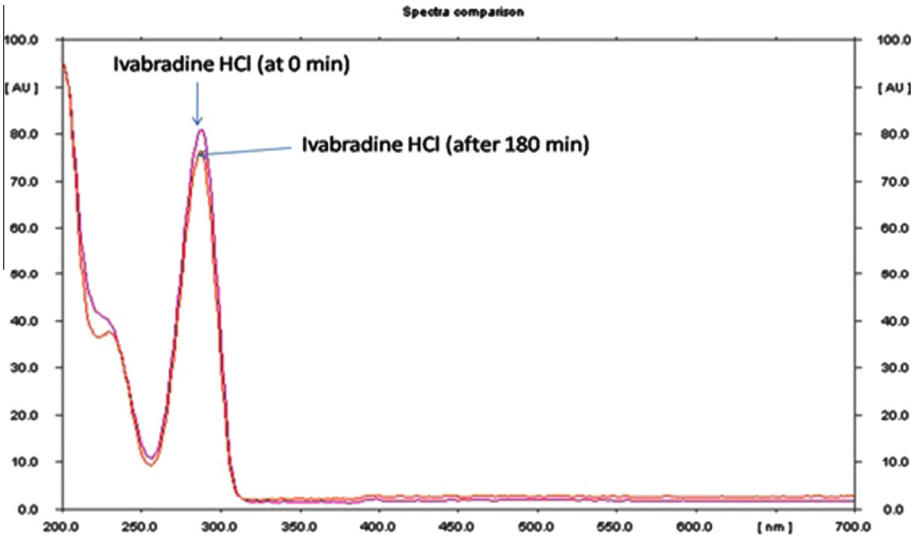

Figure 3 In situ UV overlain spectra of IH standard and IH standard after acid degradation, (——) = IH standard at 0 min, (——) = IH standard after 180 min of acid degradation.

Table 3 Analysis of IH in tablet dosage form.

Drug	Brand name	Amount present (mg per tablet)	% Amount found[a]	SD	% RSD
Ivabradine hydrochloride	Abbott	5	98.83	1.11	1.12
		7.5	101.49	0.67	0.66
	Ind swift	5	99.59	0.91	0.91
		7.5	99.62	1.33	1.34

[a] Average of three determinations for each formulation.

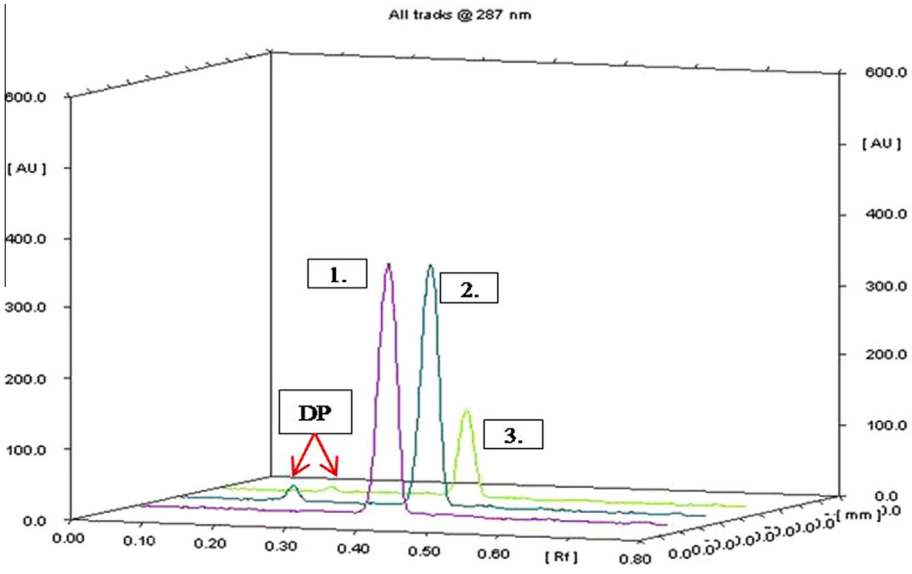

Figure 4 3D densitogram showing (1) IH standard, (2) acid degradation, (3) base degradation (R_f; IH = 0.36, DP = 0.18).

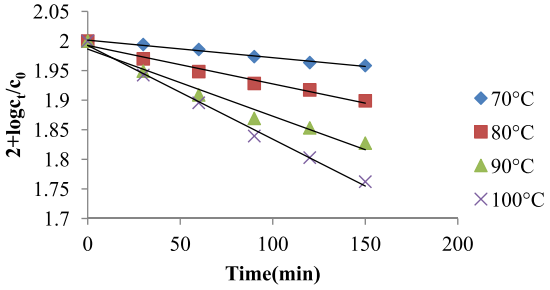

Figure 5 Degradation kinetic study showing first order plot at 70, 80, 90 and 100 °C.

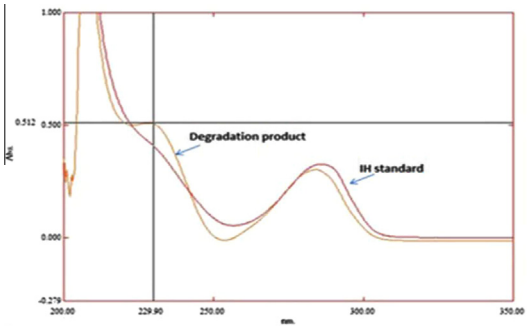

Figure 6 In situ UV overlain spectra of IH standard and IH after acid degradation (——) = IH standard, (——) = Degradation product.

Data obtained from pseudo first-order kinetics treatment was further subjected to fit in Arrhenius equation:

$$\mathrm{Log}K = \frac{\log A - E_a}{2.303\mathrm{RT}} \tag{3}$$

where, K is the rate constant, A the frequency factor (kcal mol^{-1}), E_a the energy of activation (cal mol^{-1}), R the gas constant (1.987 cal^{-1}K mol^{-1}) and T the absolute temperature (K).

A plot of (4 + log K) values versus the reciprocal temperature ($1/T \times 10^3$), the Arrhenius plot, was found to be linear in the selected temperature range (70–100 °C). An extrapolation of Arrhenius plot is used to calculate the degradation rate constants of acidic degradation process (K_{25}), activation energy (E_a), half life ($t_{1/2}$) and shelf life (t_{90}) at 25 °C. The values of all aforementioned parameters were: $K_{25} = 3.335 \times 10^{-5}\mathrm{min}^{-1}$, $E_a = 14.24$ kcal/mol, $t_{1/2} = 346.32$ h, $t_{90} = 50.97$ h.

Degradation kinetic study reveals that degradation of IH was mainly due to acid hydrolysis at higher temperature and the chromatogram shows that IH and acid degradation product (DP) were well resolved with R_f of 0.36 ± 0.02 and 0.18 ± 0.02 respectively, indicating that DP has more polar nature compared to IH. An in situ densitometric UV spectrum shows that a wavelength of maximum absorption of acid

Table 4 Comparison of proposed HPTLC method with reported HPLC method[a].

Parameters[b]	Statistical analysis
	F test (39)[c]
Intraday precision	1.431
Interday precision	6.225
Accuracy study	3.282
	T test (2.776)[c]
Intraday precision	0.763
Interday precision	1.491
Accuracy study	0.779

[a] Ref. 9.
[b] Average of three determinations.
[c] Values in parenthesis represent corresponding tabulated values of *F* and *T* at 95% confidence interval.

degradation product is similar to IH indicating a similar chromophoric structure (Fig. 6). FTIR spectra of degradation product showed similar characteristic of peaks when compared to peaks in IR spectra of IH. The peaks in IR spectra for IH standard appeared at 1644 cm^{-1} for C═O, 1100, 1300 cm^{-1} for C–O, 1439–1516 cm^{-1} for C═C aromatic ring, 1320 cm^{-1} for C–N and acid degradation product also showed similar peaks at respective positions, however, the region near 1100 cm^{-1}showed a considerable broadening and might be due to overlapping of C–O and C–N vibrations. Mass spectra of both, IH and acid degradation product when measured in positive scan ion mode showed molecular ion peak at 469 and 470 respectively, indicating similar structural mass of acid degradation product (M + 1) and IH.

4. Conclusion

A specific, precise, accurate, rapid and economic stability-indicating high-performance thin layer chromatographic method for analysis of Ivabradine hydrochloride (IH) in bulk drug and marketed formulations is developed and validated according to ICH guidelines. It is found that, IH is unstable in hydrolytic conditions only, whereas it is stable in all other conditions for specified period of time. A major advantage of developed HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering the analysis time, sample clean up and cost per analysis. Moreover, the proposed HPTLC method when compared with previously reported stability indicating HPLC method [9] for precision and accuracy study using *F* test and *T* test revealed that calculated *F* and *T* values were less than the tabulated values, stating no significant difference between two methods and good precision and accuracy of the proposed HPTLC method (Table 4). The developed method could effectively separate the drug from its degradation product; hence it can be employed as a stability-indicating one, also it is suitable for the analysis of IH in bulk and dosage form and in quality control laboratories.

5. Conflict of interest

None.

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